

Application No. 09/723,722
Amendment dated October 29, 2003
Reply to Office Action of April 29, 2003

PATENT

Amendments to the Claims:

This listing of claims will replace all prior versions, and listings of claims in the application:

Listing of Claims:

1. (Currently Amended) A ~~β -secretase enzyme protein~~ purified to apparent homogeneity comprising a segment of a β -secretase enzyme protein, wherein the segment lacks the signal sequence (amino acid residues 1-22 with respect to SEQ ID NO:2) and the putative pro region (amino acid residues 23-45 with respect to SEQ ID NO:2)~~peptide that is fewer than 460 amino acids in length, and includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 43 [46-501].~~
2. (Currently Amended) The ~~purified β -secretase enzyme protein~~ of claim 1, wherein the enzyme has been purified sufficiently so that its activity in cleaving the 695-amino acid isotype of β -amyloid precursor protein (β -APP) between amino acids 596 and 597 thereof is at least 10,000-fold greater than an activity exhibited by a solubilized but unenriched membrane fraction from human 293 cells.
3. (Currently Amended) The ~~purified β -secretase enzyme protein~~ of claim 1, characterized by a specific activity of at least about 0.2×10^5 nM/h/ μ g protein in an MBP-C125sw substrate assay.
4. (Currently Amended) The ~~purified β -secretase enzyme protein~~ of claim 3, wherein said specific activity is at least 1.0×10^5 nM/h/ μ g protein.
5. (Withdrawn) A β -secretase enzyme protein purified to apparent homogeneity comprising a peptide that is fewer than 450 amino acids in length, and includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 70 [63-452].
6. (Withdrawn) The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 70 [63-452].

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7. (Withdrawn) The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 69 [63-501].

8. (Withdrawn) The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 67 [58-501].

9. (Withdrawn) The purified protein of claim 5, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 68 [58-452].

10. (Withdrawn) The purified protein of claim 5, wherein said protein comprises a polypeptide having the amino acid sequence SEQ ID NO: 58 [46-452].

11. (Withdrawn) The purified protein of claim 10, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 74 [22-452].

12. (Withdrawn) The purified protein of claim 10, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 58 [46-452].

13. (Withdrawn) The purified protein of claim 10, wherein said protein is characterized by an N-terminus at position 46 with respect to SEQ ID NO: 2 and a C-terminus between positions 452 and 470 with respect to SEQ ID NO: 2.

14. (Canceled)

15. (Currently Amended) The ~~purified~~ protein of claim 1, wherein said protein consists of a polypeptide having the amino acid sequence SEQ ID NO: 43 [46-501].

16-17: (Canceled)

18. (Currently Amended) The ~~purified~~ protein of claim 1, wherein said protein has an N-terminal residue corresponding to a residue selected from the group consisting of residues 46, 58 and 63 with respect to SEQ ID NO: 2 and a C-terminus selected from a residue between positions 452 and 501 with respect to SEQ ID NO: 2.

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19. (Currently Amended) The ~~purified~~ protein of claim 18, wherein said C-terminus is between residue positions 452 and 470 with respect to SEQ ID NO: 2.

20. (Canceled) The purified protein of claim 1, wherein said protein is isolated from a mouse.

21. (Withdrawn) The protein of claim 20, wherein said polypeptide has the sequence SEQ ID NO: 65.

22. (Currently Amended) The ~~purified~~ protein of claim 1, wherein said protein is produced by a heterologous cell.

23. (Currently Amended) A crystalline protein composition formed from the protein of claim 1 ~~a β -secretase protein purified to apparent homogeneity comprising a peptide that is fewer than 460 amino acids in length, and includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 43 [46-501].~~

24. (Original) The crystalline protein composition of claim 23, wherein said purified protein is characterized by a binding affinity for the β -secretase inhibitor substrate P10-P4'sta D \rightarrow V which is at least 1/100 of an affinity exhibited by a protein having the amino acid sequence SEQ ID NO: 43 [46-501], when said proteins are tested for binding to said substrate under the same conditions.

25. (Original) The crystalline protein composition of claim 23, wherein said composition is formed from a protein having a sequence selected from the group consisting of SEQ ID NO: 43 [46-452], and SEQ ID NO: 71 [46-419].

26. (Canceled)

27. (Currently Amended) The crystalline protein composition of claim 23, wherein said composition is formed from a protein having an N-terminal residue corresponding to a residue selected from the group consisting of amino acid residues 58 and 63 with respect to

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SEQ ID NO: 2 and a C-terminus selected ~~from a~~ from an amino acid residue between positions 452 and 501 with respect to SEQ ID NO: 2.

28. (Original) The crystalline protein of claim 27, wherein said C-terminus is between residue positions 452 and 470 with respect to SEQ ID NO: 2.

29. (Original) The crystalline protein composition of claim 23, wherein said protein is glycosylated.

30. (Original) The crystalline protein composition of claim 23, wherein said protein is deglycosylated.

31. (Original) The crystalline protein composition of claim 23, wherein said composition further includes a β -secretase substrate or inhibitor molecule.

32. (Original) The crystalline protein composition of claim 31, wherein said β -secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence SEQ ID NO: 78 (VMXVAEF; P3-P4'X D->V), including conservative substitutions thereof.

33. (Original) The crystalline protein composition of claim 31, wherein said β -secretase inhibitor has the sequence SEQ ID NO: 72 [P10-P4'sta D->V], including conservative substitutions thereof.

34. (Original) The crystalline protein composition of claim 31, wherein said β -secretase inhibitor has the sequence SEQ ID NO: 81 [EVMXVAEF], wherein X is hydroxyethylene or statine.

35. (Original) The crystalline protein composition of claim 31, wherein said β -secretase inhibitor is characterized by a K_i of no more than about 0.5 mM.

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36. (Original) The crystalline protein composition of claim 31, wherein said β -secretase inhibitor is characterized by a K_i of no more than about 50 μ M.

37. (Withdrawn) An isolated protein, comprising a polypeptide that (i) is fewer than about 450 amino acid residues in length, (ii) includes an amino acid sequence that is at least 90% identical to SEQ ID NO: 75 [63-423] including conservative substitutions thereof, and (iii) exhibits β -secretase activity, as evidenced by an ability to cleave a substrate selected from the group consisting of the 695 amino acid isotype of beta amyloid precursor protein (β APP) between amino acids 596 and 597 thereof, MBP-C125wt and MBP-C125sw.

38. (Canceled)

39. (Withdrawn) The protein of claim 37, wherein said polypeptide has the sequence SEQ ID NO: 75 [63-423].

40. (Withdrawn) The protein of claim 37, wherein said amino acid sequence is at least 95% identical to SEQ ID NO: 58 [46-452].

41. (Withdrawn) The protein of claim 40, wherein said polypeptide has the sequence SEQ ID NO: 58 [46-452].

42. (Withdrawn) The protein of claim 37, wherein said protein consists of a polypeptide having the sequence SEQ ID NO: 58 [46-452].

43. (Withdrawn) The protein of claim 37, wherein said protein consists of a polypeptide having the sequence SEQ ID NO: 74 [22-452].

44. (Withdrawn) The protein of claim 37, wherein said protein is expressed by a heterologous cell.

45. (Withdrawn) A composition comprising the protein of claim 37 and a β -secretase substrate or inhibitor molecule.

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46. (Withdrawn) The composition of claim 45, wherein said β -secretase substrate is selected from the group consisting of MBP-C125wt, MBP-C125sw, APP, APPsw, and β -secretase-cleavable fragments thereof.

47. (Withdrawn) The composition of claim 46, wherein said β -secretase-cleavable fragment has a sequence selected from the group consisting of SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 89, SEQ ID NO: 90, SEQ ID NO: 91, SEQ ID NO: 92, SEQ ID NO: 93, SEQ ID NO: 94, SEQ ID NO: 95, and SEQ ID NO: 96.

48. (Withdrawn) The composition of claim 45, wherein said β -secretase inhibitor is a peptide having fewer than about 15 amino acids and comprises the sequence SEQ ID NO: 78 (VM[X]VAEF, where X is hydroxyethylene or statine), including conservative substitutions thereof.

49. (Withdrawn) The composition of claim 48, wherein said β -secretase inhibitor has the sequence SEQ ID NO: 81 (VM[X]VAEF, where X is hydroxyethylene or statine).

50. (Withdrawn) The composition of claim 45, wherein said β -secretase inhibitor has the sequence SEQ ID NO: 72 (P10-P4'sta D->V), including conservative substitutions thereof.

51. (Withdrawn) The composition of claim 37, wherein said β -secretase inhibitor has a K_i of no more than about 1 μ M.

52. (Withdrawn) The composition of claim 37, wherein said β -secretase inhibitor is labeled with a detectable reporter molecule.

53. (Withdrawn) An isolated mouse β -secretase protein enzyme having the sequence SEQ ID NO: 65.

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54-131: (Canceled)

132. (New) The protein of claim 1, wherein the enzyme has been purified sufficiently to run as a single band on a SDS PAGE gel under reducing conditions.

133. (New) The protein of claim 1, wherein the enzyme has been purified sufficiently to provide a suitable substrate for N-terminal amino acid determination.